Introduction
1. INTRODUCTION

1.1 Neurodegenerative disease

Neurodegenerative diseases are the heterogeneous group of disorders characterized by the progressive and selective loss of neuronal system (Lindholm et al., 2006). Brain pathology in the forms of cerebrovascular and neurodegenerative diseases are leading cause of death all over the world, with an incidence of about 8% of total death rate (Bossy-Wetzel et al., 2004; Dajas et al., 2003). According to World Health Organization about one billion people in worldwide suffer from neurological disorders and 6.8 million people die annually from these disorders. The prevalence rate of neurological disorders in India varies from 967 to 4,070 per 100,000 populations (Gourie-Devi, 2008).

1.2 Huntington’s disease

Huntington’s disease (HD) is a neurodegenerative disease characterized by the clinical triad of movement disorder, dementia and psychiatric disturbance (Hannan, 2005). The disease was initially named Huntington’s chorea after George Huntington, who wrote the first detailed description in 1872 (Huntington, 1872). The name has been changed to HD to reveal the fact that chorea is not the only important manifestation of the disease. However, the first definite description of HD by Charles Oscar Waters in 1842 provides a lucid picture of one of its main clinical features, chorea, and its hereditary nature (Water, 1842). The indications of its appearance are spasmodic twitching of the extremities, generally of the
fingers which gradually extend and involve all the involuntary muscles. HD is inherited in an autosomal dominant manner and typically develops in the fourth or fifth decade of life. Initially, patients demonstrate personality changes and develop small involuntary movements. As the disease progresses, the movement disorder become more pronounced and cognitive deficits as well as psychiatric disturbances occur. The overall prevalence of HD is 4 to 10 per 100,000 but this varies greatly between geographical regions (Leegwater-Kim and Cha, 2004).

The huntingtin gene (HTT) responsible for HD was discovered in 1993 and encodes a 350 kDa ubiquitously expressed protein called huntingtin (Htt) (Imarisio et al., 2008), which is required for neurogenesis and survival, though its functions are incompletely understood (Walker, 2007; Cattaneo et al., 2005). The causative mutation is an abnormal expansion of a tract of uninterrupted CAG (cytosine-adenine-guanosine) trinucleotide repeats within exon 1 of the Interesting Transcript 15 (IT15) gene on chromosome 4p16.3. In normal individuals, the number of CAG repeats is 35 or fewer, with 17-20 repeats found most commonly (Myers, 2004). Repeats between 27 and 35 are rare and are not associated with disease, but are meiotically unstable and can expand into the disease range of 36 and above, when transmitted through the paternal line. Most adult-onset cases have 40-50 CAGs, whereas expansions of 50 and more repeats generally cause the juvenile form of the disease. Pathologically, HD is characterized by the selective loss of efferent medium spiny neurons in the striatum (caudate nucleus and putamen) of the basal ganglia.
1.3 Pathogenesis of Huntington’s disease

The intracellular pathogenesis of HD is shown in Figure 1.1. Normal Htt is localized in cytoplasm but mutant Htt (mHtt) in addition to being found in cytoplasm is also localized in nucleus (Peters et al., 1999). The mHtt with an expanded polyglutamine repeats undergo conformational change and interfere with cellular trafficking, especially of brain-derived neurotrophic factor (BDNF) (del Toro et al., 2006). mHtt is cleaved at several points to generate toxic fragments with abnormal compact β conformation. Pathogenic species can be monomeric or more likely form small oligomers. Toxic effects of mHtt in cytoplasm include inhibition of chaperones, proteasomes and autophagy, and cause the accumulation of abnormally folded proteins (Li et al., 2010). Interaction of mHtt with mitochondria leads to adenosine triphosphate (ATP) depletion and reactive oxygen species (ROS) production (Jin and Johnson, 2010). A major action of mHtt is interference with gene transcription, in part via peroxisome proliferator-activated receptor γ, coactivator 1α (PGC1α), leading to decreased transcription of BDNF and nuclear-encoded mitochondrial proteins. mHtt also cause the dysregulation of proteins implicated in trafficking including Htt-associated protein-1 (HAP1), which in turn interacts with dynactin and affect the vesicle motility along with microtubules (Caviston et al., 2007). Nuclear localization of mHtt corresponds to increased cellular toxicity through altered protein-protein interactions, interferes with deoxyribonucleic acid (DNA) transcription and ribonucleic acid (RNA) processing, which leads to the activation of apoptosis (Li and Li, 2004; Harjes and Wanker, 2003).
1.4 **Mitochondrial dysfunction in Huntington’s disease**

The pathophysiology of HD has been linked to mitochondrial dysfunction ([Figure 1.2](#)) (Quintanilla and Johnson, 2009). Mitochondria are the vital cellular organelles that generate energy for all molecular processes and regulate cellular functions (Benard et al., 2007). Mitochondrial energy production is necessary to carry out critical cellular processes, such as neurotransmitter release and reuptake. Neurons have a high metabolic demand and energy requirements, hence they are highly susceptible to mitochondrial abnormalities (Kann and Kovacs, 2007). Mitochondrial impairment leads to increased production of ROS, and plays a central role in apoptotic cell death.
Mitochondrial dysfunction causes vulnerability to oxidative stress and the activation of downstream cell death pathways, leads to neuronal apoptosis (Facecchia et al., 2011).

Figure 1.2: Role of mitochondrial impairment in HD.


Mitochondrial dysfunction in HD may occur through aberrant transcriptional regulation, as mHtt binds to several transcriptional regulators and interferes with their function (Jin and Johnson, 2010). mHtt causes the transcriptional dysregulation of p53 and PGC-1α pathways (McGill and Beal, 2006; Bae et al., 2005). Impairment of p53 induces the expression of mitochondria associated proteins like Bcl-2–associated X protein (Bax) and p53 upregulated modulator of apoptosis (PUMA), linked to mitochondrial depolarization. Direct mHtt interactions with adenine nucleotide translocase
(ANT) and voltage-dependent anion channel (VDAC) increases the proton conductivity and promotes mitochondrial permeability transition (mPT) pore opening (Panov et al., 2005). Further, putative mHtt targets are regulatory Ca\(^{2+}\) binding sites in the mitochondrial glutamate/aspartate carrier and in the mPT pore. Evidence from HD rat brain mitochondria suggests that mHtt inhibits Ca\(^{2+}\) regulation at these sites (Gellerich et al., 2008). Inhibition at the mPT pore site might prevent a protective effect of extra mitochondrial Ca\(^{2+}\) and facilitate mPT pore opening (Gellerich et al., 2008). Soluble N-terminal mHtt disturbs the association of microtubule-based transport proteins with the mitochondria (Orr et al., 2008). Selective neuronal loss in the striatum accounts for most of the clinical features of HD (Subramaniam and Snyder, 2011). Reduced activity of the complex II enzyme succinate dehydrogenase (SDH) has been found in the post-mortem HD brain (Benchoua et al., 2006). It is suggested that an increase in polyglutamine repeats in Htt potentially inhibits the enzyme, SDH (Quintanilla and Johnson, 2009).

1.5 Clinical features

1.5.1 Motor disorder

The motor symptoms of HD include involuntary movements such as chorea and impaired voluntary movements, causes limb incoordination and impaired hand function. The pattern of symptoms tends to change over time in typical adult-onset of HD: chorea dominates in early stage of the disease, but then tends to decline as the disease progresses, with dystonia, rigidity, and bradykinesia then becoming more marked (Novak and Tabrizi, 2010). Dystonia refers to different presentations include twisting, tilting or turning of
the neck and involuntary arching of the back and feet. Juvenile-onset of HD causes sudden brief jerking of groups of muscles called myoclonus (Gambardella et al., 2001). Akathisia is an extremely uncomfortable internal sense of restlessness, which may cause patients to pace or to be unable to sit still. It can be caused by neuroleptics and can look like agitation or anxiety.

1.5.2 Cognitive disorder

Executive functions are the high-level cognitive processes that control other aspects of cognitive function, and these almost invariably decline in HD. Thinking-style becomes more concrete and less efficient, and planning, initiation and organization of time become poor, thoughts and activities become harder. HD causes delicate visuospatial perceptual changes (Paulsen et al., 2008). Memory loss and difficulty in learning new skills are common. As cognitive dysfunction progresses, patients can develop severely limiting frontal and subcortical dementia (Peavy et al., 2010).

1.5.3 Psychiatric disorder

Psychiatric symptoms are common in HD and, like cognitive symptoms, often precede motor onset by many years. Depression is extremely common in HD and occurs as an intrinsic feature of the disease (Paulsen et al., 2005). Suicide risk in HD patients is more likely than the general population to commit suicide (Hubers et al., 2012). Anxiety, irritability and agitation are general symptoms among HD patients. Obsessive-compulsive thoughts and behaviors are also relatively familiar in HD. (Paulsen et al., 2001; Rosenblatt and Leroi, 2000).
1.5.4 Other related disorders

Communication can be impaired by both dysarthria and cognitive dysfunction. Dysarthria is mainly caused by incoordination of voluntary oromotor muscle movement and, as with most other HD symptoms, is often worse when an affected individual is tired or under stress. Cognitive symptoms which contribute to communication problems include word-finding difficulties and inability to initiate or structure speech (Deckel and Cohen, 2000). Swallowing problems also arise from both motor and cognitive dysfunction (Aziz et al., 2010). Oromotor incoordination and distractibility are normal factors. Metabolic and endocrine changes are increasingly recognized in HD (Hult et al., 2010). The disease creates a catabolic state resulting in weight loss being a prominent feature of HD. Sleep disturbance is also widespread in HD (Arnulf et al., 2008).

1.6 Diagnosis

1.6.1 Genetic testing for Huntington’s disease

Genetic testing for HD is performed through quantification of the CAG repeat length in the HTT gene. Testing falls into two categories: diagnostic testing is carried out to confirm in a patient with symptoms suggestive of HD, whereas predictive testing is carried out in a person who has no symptoms of HD, but at risk because of their family history. Predictive testing determines whether an individual carries the expanded HTT gene and will develop HD in the future (Muthane, 2011).

1.6.2 Prenatal testing

Prenatal testing is usually carried out via chorionic villus sampling between 11 and 13 weeks of pregnancy. In pretest counseling, parents need to
terminate the pregnancy if their fetus is found to have an expanded HTT gene; otherwise their child will grow up in the shadow of a predictive test (Simpson et al., 2002).

1.6.3 Pre-implantation genetic diagnosis

In pre-implantation genetic diagnosis, embryos are created using normal in vitro fertilization procedure and then tested for the expanded HTT gene (Moutou et al., 2004). Unaffected embryos are implanted. Overall, about one in five cycles results in a live birth, but success rates vary.

1.6.4 Computed tomography scanning

Computer generated images of the brain’s internal structures often shown the shrinkage in two areas of the brain- the caudate nuclei and putamen in HD. Computed tomography scans combine with other procedures such as magnetic resonance imaging and/or positron emission tomography can be a helpful diagnostic tool, especially when evaluated in the context of family history and clinical symptoms (van den Bogaard et al., 2012).

1.7 Treatment

The treatment for HD focuses on reducing symptoms, preventing complications, providing support and assistance to the patient. Most of the drugs currently used for the symptomatic management of HD are discussed as follows (Ross and Tabrizi, 2011).

- Tetrabenazine and clonazepam administration suppresses the involuntary jerking and writhing movements (chorea) associated with
HD. A serious side effect is the risk of triggering depression and/or other psychiatric conditions. Other possible side effects include insomnia, drowsiness, nausea, sedation, ataxia, apathy and restlessness.

- Antipsychotic drugs, such as haloperidol, clozapine, risperidone and sulpiride are used to treat psychosis and irritability. Major side effects of these drugs include agitation, dystonia, akathisia, sedation, hypotension, constipation and increased appetite.

- Antidepressant drugs like citalopram, fluoxetine, paroxetine, sertraline, mirtazapine and venlafaxine are used to reduce depression, anxiety, obsessive compulsive behavior and irritability. These drugs as well have some side effects like gastrointestinal disturbance, hypersensitivity reactions, drowsiness, syndrome of inappropriate antidiuresis and postural hypotension.

- Anticonvulsant such as sodium valproate and levetiracetam are used to cure rigidity associated with juvenile HD. Gastrointestinal disturbance, weight gain, blood dyscrasia, hyperammonaemia and liver dysfunction are the major side effects of these drugs.

1.8 Animal models in Huntington’s disease

Although post-mortem human brain tissue from end-stage HD patients is available, animal models are precious because they provide material for histopathological, biochemical and molecular studies in the earliest stages of disease and for the assessment of cell-cell interactions (Ferrante, 2009; Wang and Qin, 2006). They are also very useful model for studying and designing
new therapeutic targets and drugs (Kim et al., 2011). The earliest animal models of HD were developed in the 1970s on the basis of selective vulnerability of striatal neurons to excitotoxic aminoacids (Coyle and Schwarcz, 1976). Other models have been developed since then: for instance, the models induced by quinolinic acid, malate and 3-nitropropionic acid (3-NP) (Kumar et al., 2010). Transgenic models have also been developed recently (Waldron-Roby et al., 2012; Farrar et al., 2011). However, the most widely used models for studying neurodegenerative processes in HD have been non-genetic models because they are easy to employ, control and acquire (Wang and Qin, 2006). In 1993, Beal et al., reported the neurochemical and histological characterization of striatal lesion produced by the mitochondrial toxin 3-NP mimicked the HD neuropathological hallmarks. Striatal neurons have also proven to be selectively vulnerable to 3-NP, suggesting that HD might disturb energy metabolism in neurons (Vis et al., 2001). For this reason, 3-NP phenotypic model is gaining attention as a valuable tool to mimic HD disorder and further developing new therapies.

1.9 3-Nitropropionic acid

![Structure of 3-Nitropropionic acid](image)

---

3-NP (Figure 1.3) is a natural environmental toxin produced by certain plant (*Indigofera endecapylla*) and fungi (*Aspergillus flavus, Astragalus and Arthriniun*). 3-NP irreversibly inhibits SDH; complex II of electron transport
chain (ETC) and tricarboxylic acid (TCA) cycle (Huang et al., 2006). The unique position of SDH on the matrix side of inner mitochondrial membrane facilitates SDH to participate in both TCA cycle and ETC. SDH catalyzes the oxidation of succinate to fumarate by reducing the flavine adenine dinucleotide (FAD) on its flavoprotein subunit. The inactivation of SDH by 3-NP is a two step process in which 3-NP is first oxidized to 3-nitroacrylate by two-electron transfer to the FAD subunit of SDH, followed by the interaction of 3-nitroacrylate with thiol group of the enzyme forming a thioether which irreversibly inactivates the SDH (Coles and Edmondson, 1979).

1.10 3-NP-induced Huntington’s disease

![Figure 1.4](image)

**Figure 1.4** : Role of 3-NP in the pathogenesis of HD.


3-NP is a complex II inhibitor of the ETC and has been found to be effectively induce selective striatal degeneration that closely replicate the
histological, neurochemical and clinical features of HD (Tsang et al., 2009; Lee et al., 2000). Oxidative stress and mitochondrial dysfunction have been projected as key events in the pathophysiology of 3-NP-induced HD (Rodríguez et al., 2010). Many studies have focused on the role of impaired energy metabolism and subsequent ROS production in HD (Mochel and Haller, 2011; Roze et al., 2008). This phenomenon is associated with reduced ATP production, later ensuing in altered calcium homeostasis, excitotoxic events, membrane depolarization and exhibits the apoptotic pathways (Figure 1.4) (Rosenstock et al., 2004).

1.11 Oxidative stress in 3-NP-induced neurodegeneration

Oxidative stress refers to the harmful effects of ROS cause damage to macromolecules such as nucleic acids, lipids, polysaccharides and proteins. The intrinsic properties of neurons like high metabolic rates, a rich composition of fatty acids prone to peroxidation, low levels of antioxidants, and reduced capability to regenerate, make them highly susceptible to the harmful effects of ROS (Reynolds et al., 2007). Free radicals alter the calcium homeostasis and ultimately resulting in the activation of neuronal nitric oxide synthase (nNOS) and subsequent release of nitric oxide (NO) (Shahani and Sawa, 2011). These events, create an imbalance between oxidant and antioxidant systems characterized by excessive production of ROS as superoxide radical (O$_2^•$), hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO$^-$) and the reduction of enzymatic and non-enzymatic antioxidant systems, resulting in oxidative stress. This phenomenon is associated with cellular damage and neuronal death, plays a crucial task in the neurodegenerative process of HD (Tasset et al., 2009; Browne, 2008).
Oxidative stress within mitochondria can lead to a vicious cycle induced by mHtt (Johri and Beal, 2012). The mHtt affects the mitochondria and produces harmful ROS, which in turn increasing the mitochondrial damage (Oliveira, 2010). Further, mHtt binds more tightly to the mitochondrial fission GTPase dynamin-related protein-1 (DRP1), causes mitochondrial fragmentation and impairs mitochondrial movement and reduces ATP (Milakovic and Johnson, 2005). mHtt interferes with the transcription of genes involved in mitochondrial biogenesis and antioxidant defense. Studies in HD patients and experimental models of HD support the oxidative stress mediated neuronal degeneration (Stack et al., 2008; Beal and Ferrante, 2004). Markers of oxidative damage include malondialdehyde (MDA) and carbonyl has elevated in human HD striatum (Tunez et al., 2011). Recently, it has shown that the oxidation of mitochondrial enzymes result in decreased catalytic activity in the striatum samples of HD patients which provide a link to the bioenergetic deficits observed in HD (Sorolla et al., 2010). Many of the oxidative alterations observed in human HD are recapitulated in neurotoxin rodent models of HD (Stack et al., 2008; Browne and Beal, 2006).

1.12 Inflammation in 3-NP-induced neurodegeneration

Inflammation is a defense reaction against various insults, intended to remove noxious agents and to inhibit their harmful effects. It consists of an incredible array of cellular and molecular mechanisms and an intricate network to control and to keep them in check. Inflammation in neurodegenerative diseases is triggered by the accumulation of proteins with abnormal conformations or by signals emanating from injured neurons. Altered expressions of different inflammatory factors can promote
neurodegenerative processes (Wyss-Coray and Mucke, 2002). Cytokines and chemokines are the key regulators of inflammatory processes and have been implicated in the pathogenesis of neuroinflammation (Lee et al., 2002).

**Figure 1.5:** Immune response in HD.
**Source:** Journal of Experimental Medicine, (2008), 205, 1869-1877.

Persistent neuroinflammatory processes contribute to the cascade of events culminating in progressive neuronal damage observed in many neurodegenerative disorders (Amor et al., 2010). The nature of immune activation in HD remains incompletely explored. A cell-autonomous effect of the mutant protein may be responsible for innate immune response (Godavarthi et al., 2009). Nuclear factor-kappa B (NF-κB) is a redox-sensitive transcription factor that governs the gene expression involved in the inflammatory processes (Li and Verma, 2002). The NF-κB signaling pathway triggers interleukin-6/8 (IL-6/8) and tumor necrosis factor-alpha (TNF-α).
Their release are known to be up-regulated by mHtt and microglia-derived toxicity, further influence disease progression and contribute to neuroinflammation (Khoshnan and Patterson, 2011; Shin et al., 2005). The inflammation associated with 3-NP, also acts as a foremost causative factor for neuronal damage in neurodegenerative disorders (Moller, 2010). Oxidative stress elicited by 3-NP activates the transcription of numerous inflammatory genes (Kumar et al., 2011). Inflammation in HD is mediated by soluble pro-inflammatory molecules such as cytokines, prostaglandins and NO (Figure 1.5) (Ahuja et al., 2008; Lee et al., 2008). Excessive release of NO is correlated with the progression of neurodegenerative disorders. Glial fibrillary acidic protein (GFAP) expression, a well recognized marker of glial cell activation is also up-regulated in neuroinflammatory conditions (Middeldorp and Hol, 2011). 3-NP induces neuroinflammation with increase in the expressions of TNF-α, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and GFAP (Kumar et al., 2011).

1.13 Role of nitric oxide in Huntington’s disease

Nitric oxide (NO) is an omnipresent intercellular messenger produced from amino acid L-arginine by the members of NO synthase (NOS) family proteins. In central nervous system (CNS), NO production is associated with cognitive function, its role is spanning from the induction and maintenance of synaptic plasticity to the control of sleep, appetite, body temperature and neurotransmission (Guix et al., 2005; Rivier, 2001; McCann, 1997). NO is produced by all brain cells including neurons, endothelial cells and glial cells (astrocytes, oligodendrocytes and microglia). Physiologically NOS in neurons (nNOS, type I NOS) and in endothelial cells (eNOS, type III NOS) produce
nanomolar amounts of NO to manage the normal neuroendocrine function (Moncada and Bolanos, 2006; Szabo, 1996). Consequently, NO plays an important role in amplifying toxicity in CNS, where large amounts of NO are produced in the brain as a result of induced expression of iNOS (type II NOS) in glial cells, phagocytes and vascular cells (Bachschmid et al., 2003) and mediating tissue damage (Bishop and Anderson, 2005). Increased oxidative stress and excessive production of NO contribute to the development of HD by damaging neighboring neurons (Butterfield et al., 2001). Further, the levels of 3-Nitrotyrosine (3-NT) was increased in brain during HD (Perez-Severiano et al., 2002). Importantly, both NOS inhibitors and ONOO\(^-\) scavengers decreased neuronal damage, reduced disease progression and also decreased brain 3-NT content in experimental models (Perez-De La Cruz et al., 2005; Santiago-Lopez et al., 2004; Deckel et al., 2001).

Figure 1.6: Molecular mechanisms of peroxynitrite-mediated cell death.

3-NP also induces the release of reactive molecules deriving from NO through stimulation of NOS activity (Deshpande et al., 2006). Thus, subsequent induction of NOS leads to NO production. In turn, NO may react with $\text{O}_2^{•−}$ to produce ONOO$^{•−}$. The later molecule is characterized by its high cytotoxicity and its capability to induce lipid peroxidation, protein nitration and oxidation, DNA oxidative damage, activation of matrix metalloproteinases (MMP) and inactivation of mitochondrial enzymes. It leads to reduced ATP formation, which dissipates the mitochondrial membrane potential ($\Delta\psi$). These events result in mitochondrial swelling, permeabilization of the outer mitochondrial membrane, allowing the efflux of several proapoptotic molecules, including cytochrome $c$ and apoptosis-inducing factor (AIF). In addition to its damaging effects on mitochondria, ONOO$^{•−}$ inflicts severe oxidative injury to DNA, resulting in DNA strand breakage which in turn activates the nuclear enzyme poly(ADP-ribose) polymerase (PARP). Activated PARP amplify the mitochondrial efflux of AIF (Figure 1.6) (Pacher et al., 2007).

1.14 Role of matrix metalloproteinases in neurodegeneration

The brain accounts for ~2% of total body mass, but it requires ~20% of total blood flow from heart to supply the cells of brain with continual fresh oxygen and energy for the maintenance of homeostasis (Shulman et al., 2004). The exquisite vascular network within the brain accomplishes this delivery of oxygen and gasses, as well as the removal of numerous potentially toxic molecules, via a continual and seamless perfusion of the entire brain (Lochhead et al., 2010). The selectivity of the blood brain barrier (BBB)
allows some molecules to move between the peripheral circulation into brain, and vice versa, all the while assisting in maintaining homeostasis of neurons, glia and vascular cells in the immediate area of BBB. One of the well-studied mechanisms for disruption of BBB by oxidative stress is via matrix metalloproteinase (MMP) activation (Grammas et al., 2011).

MMPs are the large family of calcium-dependent zinc-containing endopeptidases, responsible for tissue remodeling and degradation of the extracellular matrix (ECM) (Page-McCaw et al., 2007). MMPs can catalyze the degradation of all protein constituents of the ECM, and their activities are kept under tight control to prevent tissue destruction. MMPs are divided into four main subgroups on the basis of domain structure: collagenases, gelatinases, stromelysins and membrane-type MMPs (MT-MMPs). The interstitial collagenases (MMP-1, -8 and -13), that preferentially have affinities toward collagen types I, II and III, the stromelysins (MMP-3, -10 and -11) have specificity for laminin, fibronectin and proteoglycans, and the gelatinases (MMP-2 and -9), which most effectively cleave type IV and V collagen. In the brain, gelatinases A (MMP2) and B (MMP9) have studied most intensively because of their prominent role in neurodegeneration (Rosenberg, 2009). Gelatinases degrade molecules in the basal lamina around capillaries, facilitate angiogenesis and neurogenesis, and contribute to instigating cell death (Gasche et al., 2006). The activities of MMPs are regulated in three ways: gene transcription, pro-enzyme activation and by the action of tissue inhibitors of metalloproteinases (TIMPs) (Clark et al., 2007). The four known TIMPs share many properties but also have distinct activities. TIMPs form complexes with the inactive pro-enzyme forms of gelatinases.
These complexes, such as pro-MMP-9/TIMP-1, pro-MMP-2/TIMP-2 and pro-MMP-2/TIMP-3 complexes, control the rate at which physiological factors activate MMPs (Nagase et al., 2006). Therefore, a favorable balance between MMPs and TIMPs is essential for preventing pathological conditions (Gardner and Ghorpade, 2003).

BBB dysfunction is a possible mechanism involved in the progressive striatal damage induced by 3-NP (Duran-Vilaregut et al., 2011). MMPs are the specific mediators of BBB disruption that incriminate oxidative stress. Among MMPs, MMP2 and MMP9 are able to digest endothelial basal lamina and lead to the opening of BBB (Rosenberg et al., 1998). MMP activity is significantly elevated in mouse models of HD and reduced MMP activity suppresses mHtt-induced neuronal dysfunction. In striatal neurons, MMP may process mHtt into a toxic fragment intracellularly and possibly processing other cellular substrates (Miller et al., 2010). It has been reported that ROS production followed by MMP activation and subsequent BBB disruption take part in the pathophysiological responsibility in 3-NP-induced neurodegeneration (Kim et al., 2003). Considering the imperative part of MMPs in neurodegenerative disorders, it is indispensable to develop new beneficial strategies targeting this family of enzymes.

1.15 Apoptosis in Huntington’s disease

Apoptosis contribute a vital role for the maintenance of homeostasis in most of the multicellular organisms (Smaili et al., 2003). On the biochemical level, two apoptotic cascades have been described: the intrinsic and extrinsic pathways. The intrinsic pathway is predominant in neurons and is decisively mediated by mitochondria (Mattson et al., 2008). This type of cell death can be
triggered by the activation of certain death receptors or by cellular stress (Wang, 2001) that involves cascades of biochemical events tightly regulated. The mitochondrial death pathway is regulated by a fine balance between pro-apoptotic and pro-survival B-cell lymphoma-2 (Bcl-2) family members (Cory and Adams, 2002). Pro-apoptotic protein, Bax can disrupt outer mitochondrial membrane and promote the release of apoptogenic factor cytochrome c, an event that ultimately activates the caspase cascade.

In normal conditions, pro-apoptotic proteins are generally repressed by binding to pro-survival proteins such as Bcl-2 and B-cell lymphoma-extra large (Bcl-xL). In response to stress, Bcl-2-associated agonist of cell death (Bad) binds to Bcl-2 or Bcl-xL, and release Bax. The pro-apoptotic protein, Bax then changes their conformation and oligomerize in the mitochondrial membrane, this directs to the release of cytochrome c into the cytosol, where it binds to the adapter protein apoptotic protease activating factor-1 (Apaf-1), promoting caspase cascade that leads to cell death. The activation of apoptotic signal transduction and subsequent cell death may be critical in selective striatal degeneration induced by 3-NP (Kim et al., 2000; Sato et al., 1997). Moreover in human HD striatal tissue, activation of pro-apoptotic proteins such as Bax, caspase and release of cytochrome c have been demonstrated (Vis et al., 2005). It has been reported that prevention of apoptosis is associated with the up-regulation of Bcl-2 and the down-regulation of Bax (Shihab et al., 1999; Wang et al., 1999). The induction of apoptosis is presumably related to oxidative stress mediated mitochondrial dysfunction (Rosenstock et al., 2004). Prevention of ROS induced oxidation protects neurons from apoptosis and delays or prevents the progression of neurodegeneration.
1.16 **Involvement of heat shock proteins in neuroprotection**

Heat shock proteins (Hsps) are the highly conserved, ubiquitously expressed family of stress response proteins which are expressed at low levels under normal physiological conditions. Hsps can function as molecular chaperones, facilitating protein folding, preventing protein aggregation or targeting improperly folded proteins to specific degradative pathways (Haslbeck *et al.*, 2005). The superfamily of heat shock proteins is divided into five major classes, four of them with a molecular mass of 60 kDa, 70 kDa, 90 kDa and 100 kDa, and small heat shock proteins with molecular mass ranging from 12 kDa to 43 kDa (Haslbeck *et al.*, 2008). In response to cellular stress, such as hyperthermia, oxidative damage, physical injury and/or chemical stressors, the expression of Hsps increases dramatically (Richter *et al.*, 2010). In general, coordinated activation of Hsp expression is called as the heat shock response (HSR). Hsps perform versatile actions in cells, from housekeeping duties under unstressed conditions, including regulation of protein quality control, to life and death decisions following cellular stress, via their interaction with members of the apoptotic cell death cascade (Lanneau *et al.*, 2008).

Increasing evidence underscores the high potential of the Hsp system as a target for new neuroprotective strategies, especially those aimed at minimizing deleterious consequences associated with oxidative stress in neurodegenerative disorders and brain aging (Sajjad *et al.*, 2010; Kalmar and Greensmith, 2009). Induction of Hsps has been shown to be protective in neurodegenerative diseases including HD (Adachi *et al.*, 2009; Guzhova *et al.*, 2011). Hsps exert its protection primarily by inhibiting the
neuroinflammation and apoptosis (Read and Gorman, 2009). Among several multi-member families of Hsps, Hsp27 and Hsp70 are the best characterized endogenous factors involved in protecting brain from various pathological conditions (Galli *et al*., 2006; Latchman, 2005). The small Hsp27 is well documented to promote neuronal survival in neurodegenerative diseases (Read and Gorman, 2009). Hsp27 can directly inhibit apoptotic pathways, and as a chaperone it can ameliorate the toxic effects of misfolded proteins in HD (Concannon *et al*., 2003). Hsp27 decreased ROS in cells expressing mHtt, suggesting that Hsp27 chaperone protects cells against oxidative stress (Wyttenbach *et al*., 2002). Another Hsp involved in the amelioration of neurodegeneration is Hsp70, which has recently been identified as potent modulator of mHtt aggregation and/or cell death in HD (Guzhova *et al*., 2011). Hsp70 has been found to reduce the expression of COX-2 and NO level (Ialenti *et al*., 2005; Van Molle *et al*., 2002). These anti-inflammatory actions of Hsp70 are mediated by binding of Hsp70 to NF-κB and its subsequent inhibition (Zheng *et al*., 2008; Jo *et al*., 2006). Hence, Hsp27 and Hsp70 appear to play a major role as therapeutic targets for HD.

1.17 Brain derived neurotrophic factor/Tyrosine receptor kinase B signaling cascade

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor ubiquitously and most abundantly expressed in the mammalian brain. It promotes the growth, development, differentiation and maintenance of neuronal system, neuronal plasticity, synaptic activity and neurotransmitter-mediated activities (Binder and Scharfman, 2004; Chao, 2003). BDNF is primarily synthesized as pro-BDNF, a precursor protein that is cleaved in the
trans-golgi or in secretory granules at highly conserved dibasic amino acid cleavage site, to generate the matured biologically active BDNF protein. BDNF is widely distributed in the CNS, with higher levels in the cerebral cortex, basal forebrain, striatum, hippocampus, hypothalamus, brainstem and cerebellum (Murer et al., 2001; Hofer et al., 1990).

BDNF exerts biological effects on the neuronal system through binding with specific transmembrane receptor, tyrosine receptor kinase B (TrkB) (Patapoutian and Reichardt, 2001). The binding of BDNF to TrkB, leads to the dimerization and autophosphorylation of tyrosine residues in the intracellular domain of the receptor (Numakawa et al., 2010). The phosphotyrosine residues of TrkB receptor function as binding sites for cytoplasmic Src homology domain-containing adaptor protein (SHC) results in recruitment of growth factor receptor-bound protein-2 (GRB2) and the Ras exchange factor of SOS to the membrane, thereby stimulating transient activation of Ras. In turn, Ras activates phosphoinositide 3-kinases (PI-3K)/Akt, mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK1/2) pathway (Kumamaru et al., 2011). PI3K can also be activated through the adaptor protein, GRB2-associated binding protein-1 (GAB-1). Another signaling pathway activated by TRK involves phospholipase C-γ resulting in inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG)-mediated signals. IP3 causes an increase in Ca^{2+} through its release from intracellular stores. DAG activates protein kinase-C (PKC), increasing the sensitivity of the contractile apparatus to Ca^{2+} as well as inducing intracellular signaling mechanisms that promote long-term cellular responses. The activation of PI-3K/Akt signaling pathway reduces the onset of apoptosis (Sabbatini and McCormick, 1999).
1.18 Role of BDNF/TrkB in Huntington’s disease

BDNF has been suggested as a therapeutic candidate to treat neurodegenerative disorders because they promote neuronal survival (Zuccato and Cattaneo, 2009). BDNF is predominantly relevant in HD since its transcription is decreased by the presence of mHtt, affecting the survival of both striatal and cortical neurons (Zuccato and Cattaneo, 2007). Reduced BDNF level has also been observed in genetic models of HD (Diekmann et al., 2009). A number of studies have shown that BDNF is a potent neurotrophic factor for GABA-ergic striatal neurons (Cepeda et al., 2004). mHtt has the ability to affect TrkB level in HD, and decreased striatal TrkB protein levels are associated with reduced mRNA expression (Gines et al., 2006). In addition, TrkB expression seemed to depend on the length of CAG tract in HTT gene, further confirming that poly Q-dependent mechanism may underlie the decrease in TrkB expression (Gines et al., 2006). BDNF was also reported to protect striatal neurons from 3-NP-induced neurodegeneration (Wu et al., 2010). Accordingly, the neuroactive compound that could stimulate brain secretion of these neurotrophic factors could be beneficial in the development of therapeutic strategies for HD.

1.19 PI-3K/Akt signaling in Huntington’s disease

BDNF exerts its biological function through binding to its receptor, TrkB, which initiates PI-3K/Akt signaling cascade (Nguyen et al., 2009). The PI-3K/Akt signal transduction pathway is important in mitogenic signaling, cell survival, growth and motility (Krasilnikov, 2000). At the plasma membrane, PI-3K catalyses the phosphorylation of phosphoinositides on the
3′-OH position of the inositol ring to generate 3′-phosphorylated phosphoinositides (3-PIs) as second messengers (Engelman et al., 2006; Hawkins et al., 2006). The 3-PIs bind to the pleckstrin homology domain-containing proteins such as Akt in order to activate this protein (Zhang et al., 2007). Akt, a serine-threonine kinase also known as protein kinase B (PKB) is a potent pro-survival kinase that exerts its survival effect in neurons by phosphorylating several substrates (Franke et al., 2003). One of the first targets of Akt in regulating cell survival is the pro-apoptotic Bad protein. It exemplified the molecular pathways linking survival factor signaling to apoptotic suppression (Song et al., 2005). The function of Akt extends beyond maintaining mitochondrial integrity to keep cytochrome c and other apoptogenic factors in the mitochondria (Kennedy et al., 1999). Akt activity also mitigates the response of cells for the release of cytochrome c into the cytoplasm. The Akt signaling pathway is of particular interest in the case of neurodegenerative disorders (Burke, 2007). In HD, Akt phosphorylates mHtt and this phosphorylation abrogates its toxicity (Humbert et al., 2002). Consequently, studying the PI-3K/Akt intracellular signaling pathway could help to elucidate the cellular mechanisms that control the toxicity in HD.

1.20 Nrf2 signaling cascade in neuroprotection

Reactive electrophiles produced during pathological processes disturb the physiological function of cellular macromolecules such as DNA, protein, or lipids and contribute to the pathogenesis of various diseases including neurodegenerative diseases (Schnabel and Blankenberg, 2007; Trushina and McMurray, 2007; Tian et al., 1998). To counteract these insults, cells have acquired an intricate mechanism of defense against toxicity. A battery of
genes encoding detoxifying and anti-oxidative stress enzymes/proteins are coordinately induced on exposure to electrophiles and ROS (Limon-Pacheco and Gonsebatt, 2009). This coordinated response is regulated through a cis-acting element called the antioxidant-responsive element (ARE) (Lyakhovich et al., 2006).

**Figure 1.7** : Nrf2 signaling pathway.

**Source** : Pharmacological Research, (2008), 58, 262-270.

Nuclear factor-erythroid 2-related factor-2 (Nrf2), a basic leucine zipper transcription factor regulates the gene expression of phase II detoxification and antioxidant enzymes such as NAD(P)H: quinone oxidoreductase-1 (NQO-1), glutathione S-transferases (GSTs), heme oxygenase-1 (HO-1) and gamma-glutamylcysteine ligase (γ-GCL) through an promoter sequence referred to as ARE (Lee and Johnson, 2004). Nrf2 under the normal quiescent cellular environment is regulated by its cytosolic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), which is anchored to the actin cytoskeleton. Keap1 acts as
a substrate adaptor protein for cullin-3-dependent ubiquitination of Nrf2, which leads to rapid Nrf2 degradation by the proteosome system. Upon activation, Nrf2 is released from Keap1 and translocates to the nucleus in heterodimeric combinations with other transcription factor Maf, and binds to the 5′-upstream regulatory ARE regions of phase II and antioxidant genes and accelerates their transcription (Figure 1.7) (Nguyen et al., 2004).

NQO-1 is an enzyme that catalyses the reduction of quinones to hydroquinones, thereby preventing the one electron reduction of quinones that would otherwise produce ROS within the cell (Kaspar et al., 2009). HO-1 oxidatively cleaves heme to biliverdin, forms carbon monoxide and releases the chelated Fe$^{2+}$. Bilirubin is a reduction product of biliverdin, serves as potent radical scavenger and protects neuronal cells against oxidative stress (Gozzelino et al., 2010). GST is a phase II enzyme, detoxifies metabolites of xenobiotics as well as reactive carbonyls, epoxides and hydroperoxides (Maher, 2005). GST was proposed to be an important defense mechanism against ROS-induced neurological disorders (Smeyne et al., 2007). γ-GCL catalyses the rate-limiting ATP-dependent step in glutathione synthesis, the formation of γ-glutamine-cysteine from glutamine and cysteine (Toroser et al., 2006). The facts that ROS lead to neurodegeneration and antioxidant therapy has neuroprotective effects in HD may point to a potential beneficial effect of the Nrf2-ARE pathway. Studies suggest that the activation of Nrf2 can confer significant protection to neurons and drugs that activate this pathway may have efficacy in blocking neuronal cell death (Calkins et al., 2009; Johnson et al., 2008). Indeed, a current work has shown that the treatment with flavonoids could activate the Nrf2-ARE pathway and confer protection against neurodegenerative diseases (Scapagnini et al., 2011).
1.21 Flavonoids

The role of natural compounds, such as flavonoids has gained enormous attention in the treatment of neurodegenerative diseases (Zeng et al., 2010; Zhao, 2005). Flavonoids comprise the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Major dietary sources of flavonoids include fruits, vegetables, cereals and tea (D'Archivio et al., 2007). Flavonoids consist of two aromatic carbon rings, benzopyran (A and C rings) and benzene (B ring), and may be divided into various sub-groups based on the degree of oxidation in C-ring and the hydroxylation pattern of the ring structure and the substitution of the 3-position. The main dietary groups of flavonoids are flavonols, which are found in onions, leeks, broccoli; flavones, which are found in parsley and celery; isoflavones, which are mainly found in soy and soy products; flavanones, which are mainly present in citrus fruit and tomatoes; flavanols, which are abundant in green tea, red wine, chocolate; and anthocyanidins, whose sources include red wine and berry fruits. In general, they are hydroxylated, methoxylated and/or glycosylated derivatives. The linked sugar is often glucose or rhamnose (Bravo, 1998).

Flavonoids have been proposed to exert beneficial effects in multitude of disease states, including cancer, cardiovascular disease and neurodegenerative disorders (Vauzour et al., 2008; Middleton et al., 2000). Many of the biological actions of flavonoids have been attributed to their antioxidant properties, either through their reducing capacities or through their possible influences on intracellular redox status (Pietta, 2000). Their antioxidant activity is associated with the number of \( \cdot \)OH groups. Because of
the high reactivity of the \textsuperscript{•}OH group of the flavonoids, radicals are made inactive (Nijveldt \textit{et al.}, 2001). Flavonoids also exerts modulatory effect in cells independent of classical antioxidant capacity through selective actions at different components of number of protein kinase and lipid kinase signaling cascades such as PI 3-kinase, Akt/PKB, tyrosine kinases, PKC and MAP kinases. Inhibitory or stimulatory actions at these pathways are likely to profoundly affect cellular function by altering the phosphorylation state of target molecules or by modulating gene expression (Williams \textit{et al.}, 2004).

1.22 Naringin

![Figure 1.8: Structure of naringin](image)

Figure 1.8: Structure of naringin

Naringin (4', 5, 7-trihydroxy-flavanone-7-rhamnoglucoside) (Figure 1.8), a flavanone presents in grapefruit and related citrus species, which is responsible for the sour flavor. When naringin is administrated orally, it is hydrolyzed by the enzymes \(\alpha\)-rhamnosidase and \(\beta\)-glucosidase to yield a major metabolite-naringenin (Kim \textit{et al.}, 1998). Naringin was reported to be nontoxic upto 1,250 mg/kg b.w (Liu \textit{et al.}, 2011). Naringin had recently received significant attention as dietary antioxidant. Naringin is known to have a broad spectrum of pharmacological and therapeutic properties like antioxidant, lipid lowering and metal chelating effects (Jagetia and Reddy, 2011; Jung \textit{et al.}, 2003). Recently, naringin has been demonstrated to play an
imperative function as an antioxidant by up-regulating the gene expressions of SOD, CAT and GPx (Jeon et al., 2001). Besides, naringin possess antimicrobial, antimitagenic, anticancer and anti-inflammatory effects (Tsui et al., 2008; Kawaguchi et al., 2011; Attia, 2008; Kanno et al., 2005). It has recently received considerable attention as a neuroprotective agent (Kim et al., 2009). Several studies have investigated the potential beneficial effects of naringin on CNS diseases (Kumar and Kumar, 2010; Gaur et al., 2009). Furthermore, the mechanisms underlying neuroprotective role of naringin are not fully understood.

The objective of this study is to elucidate the protective effect of naringin on 3-NP-induced neurodegeneration through the modulation of BDNF/TrkB and Nrf2 signaling cascades.

References


Coyle, J.T., Schwarz, R., 1976. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature. 263, 244-246.


Lee, W.T., Shen, Y.Z., Chang, C., 2000. Neuroprotective effect of lamotrigine and MK-801 on rat brain lesions induced by 3-nitropropionic acid: evaluation by magnetic resonance imaging and in vivo proton magnetic resonance spectroscopy. *Neurosci.* 95, 89-95


Milakovic, T., Johnson, G.V., 2005. Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. J. Biol. Chem. 280, 30773-30782.


Subramaniam, S., Snyder, S.H., 2011. Huntington's disease is a disorder of the corpus striatum: focus on Rhes (Ras homologue enriched in the striatum). Neuropharmacol. 60, 1187-1192.


